

to amplify polyketide synthetase type I genes (Hutchinson *et al.*, *Annual Review of Microbiology*, 1995, **49**:201-238). PCR primers PR144 and PR145 were used to amplify a 0.6 kb fragment from *M. carbonacea* chromosomal DNA. The 0.6 kb fragment was cloned into the pNOTA vector (5 Prime 3 Prime Inc., Boulder, Co) and sequence analysis of the insert revealed BLAST homology to polyketide type I genes. PCR analysis of the *M. carbonacea* cosmid library using PR144 and PR145 were used to isolate cosmid pSPR150. The 4 kb pSPR150 insert was sequenced and revealed numerous phage like genes including genes and DNA regions with homology to database integrases, excisionases and *attP* attachment sites--

Please replace the first full paragraph of page 23 with the following paragraph:

--PCR primers PDH504 (5' AGGGCAACAAGGGAAGCGTC 3') (SEQ ID NO: 13) and PDH505 (5' GGCGGGGGTGTGGCTATTATT 3') (SEQ ID NO: 14) were designed to amplify the *attB* region from *M. carbonacea*. PCR amplification of *M. carbonacea* chromosomal DNA yielded a fragment with homology to tRNA-His (bp 45...119, Fig. 4b). Contained within this tRNA-His gene, at the 3' end, is the *M. carbonacea* *attB* site (bp 95...119, Fig. 4b) that has perfect homology to the pMLP1 *attP* site (bp 101...125, Fig. 4a). PCR primers PDH 502 (5' TTGTTGGTCCGGCCCCAACG 3') were designed to amplify the *attB* region from *M. halophitica*. PCR amplification of *M. halophitica* chromosomal DNA yielded a fragment with homology to tRNA-His (bp 45...120, Fig. 5b). Contained within this tRNA-His gene, at the 3' end, is the *M. halophitica* *attB* site (bp 96...121, Fig. 5b) that has perfect homology to the pMLP1 *attP* site (bp 101...125, Fig. 5a).--

Please delete the Sequence Listing and insert the enclosed substitute Sequence Listing into the specification after the ABSTRACT.

REMARKS

Entry of the foregoing Amendment is requested. As of entry of the Amendment, claims 1-4, 6, 8-18 and 20-22 will be pending.

No new matter was added to the Application. Written support for amended claim 22 appears in the specification, for example, at figure 4, first three lines. Moreover, the

Amendment only makes formal changes to the specification which, again, does not add new matter to the Application.

Response to the Notice to Comply with the Sequence Rules

Applicants submit that the enclosed substitute Sequence Listing sets forth all nucleotide and amino acid sequences appearing in the specification.

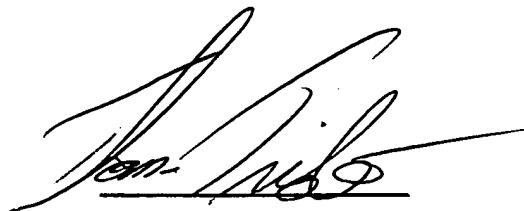
Statement Under 37 C.F.R. §1.821

The content of the attached paper entitled "SEQUENCE LISTING" and of the accompanying identically labeled diskette, specifically the text file therein labeled "seqlist.txt", is the same. Furthermore, the information contained in the attached "SEQUENCE LISTING" and the text file is identical to the information originally submitted with the specification. Accordingly, no new matter has been added to the Application.

CONCLUSION

Early and favorable action is earnestly solicited.

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Hosted, *et al.*

Serial No.: 09/855,340

Group Art Unit: 1645

Filed: May 15, 2001

Examiner: To be assigned

For: ISOLATION OF MICROMONOSPORA CARBONACEA VAR AFRICANA PMLP1
INTEGRASE AND USE . . .

Confirmation No. 9296

MARKED-UP AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

The following Marked-Up Amendment shows all changes which are made by the enclosed Preliminary Amendment.

In the claims: Please amend claim 22 as follows:

22 (Twice amended). An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: [4-9] 4-10.

In the specification: Please replace the third full paragraph of page 13 with the following paragraph:

- -Degenerate PCR primers PR144 (5' TGCTTCGACGCCATCARGG3') (SEQ ID NO: 11) and PR145 (5'GTGGAAICCGCCGAAKCCGC3') (SEQ ID NO: 12) were designed to amplify polyketide synthetase type I genes (Hutchinson *et al.*, *Annual Review of Microbiology*, 1995, **49**:201-238). PCR primers PR144 and PR145 were used to amplify a 0.6 kb fragment from *M. carbonacea* chromosomal DNA. The 0.6 kb fragment was cloned

into the pNOTA vector (5 Prime 3 Prime Inc., Boulder, Co) and sequence analysis of the insert revealed BLAST homology to polyketide type I genes. PCR analysis of the *M. carbonacea* cosmid library using PR144 and PR145 were used to isolate cosmid pSPR150. The 4 kb pSPR150 insert was sequenced and revealed numerous phage like genes including genes and DNA regions with homology to database integrases, excisionases and *attP* attachment sites--

Please replace the first full paragraph of page 23 with the following paragraph:

- -PCR primers PDH504 (5' AGGGCAACAAGGGAAGCGTC 3') (SEQ ID NO: 13) and PDH505 (5' GGCGGGGTGTGGCTATTATT 3') (SEQ ID NO: 14) were designed to amplify the *attB* region from *M. carbonacea*. PCR amplification of *M. carbonacea* chromosomal DNA yielded a fragment with homology to tRNA-His (bp 45. . .119, Fig. 4b). Contained within this tRNA-His gene, at the 3' end, is the *M. carbonacea* *attB* site (bp 95. . .119, Fig. 4b) that has perfect homology to the pMLP1 *attP* site (bp 101. . .125, Fig. 4a). PCR primers PDH 502 (5' TTGTTGGTCCGGCCCCAACG 3') were designed to amplify the *attB* region from *M. halophitica*. PCR amplification of *M. halophitica* chromosomal DNA yielded a fragment with homology to tRNA-His (bp 45. . .120, Fig. 5b). Contained within this tRNA-His gene, at the 3' end, is the *M. halophitica* *attB* site (bp 96. . .121, Fig. 5b) that has perfect homology to the pMLP1 *attP* site (bp 101. . .125, Fig. 5a).--